SUPPLEMENTAL MATERIAL

STING-IRF3 Triggers Endothelial Inflammation in Response to Free Fatty Acid-Induced Mitochondrial Damage in Diet-Induced Obesity

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Supplemental Figure Legends：

Figure S Ⅰ. PA induced STING-IRF3 activation and ICAM-1 expression to a similar extent and in a similar pattern in HAECs from different donors. Two batches of HAECs from different donors were treated with PA for 24 hours. **A,** Western blot results indicated that PA induced IRF3 phosphorylation and ICAM-1 expression similarly in the 2 batches of cells. **B-C**, Immunostaining showed that PA induced STING perinuclear translocation **(B)** and IRF3 nuclear translocation **(C)** in both batches of cells**.**

Figure S Ⅱ. The STING-IRF3 pathway was activated in the aortas of obese mice.

Wild-type mice were fed either a chow diet (CD) or high-fat diet (HFD) for 12 weeks. **A,** The body weights and free fatty acid (FFA) levels of the 2 groups were compared. **B,** Western blot analysis showed increases in the levels of total IRF3, phospho-IRF3 and ICAM-1 in the aorta of the HFD-fed mice. **C-D,** Immunostaining showed that STING **(C)** and IRF3 **(D)** translocation were increased in the aortic wall of the HFD-fed mice.

Figure S I

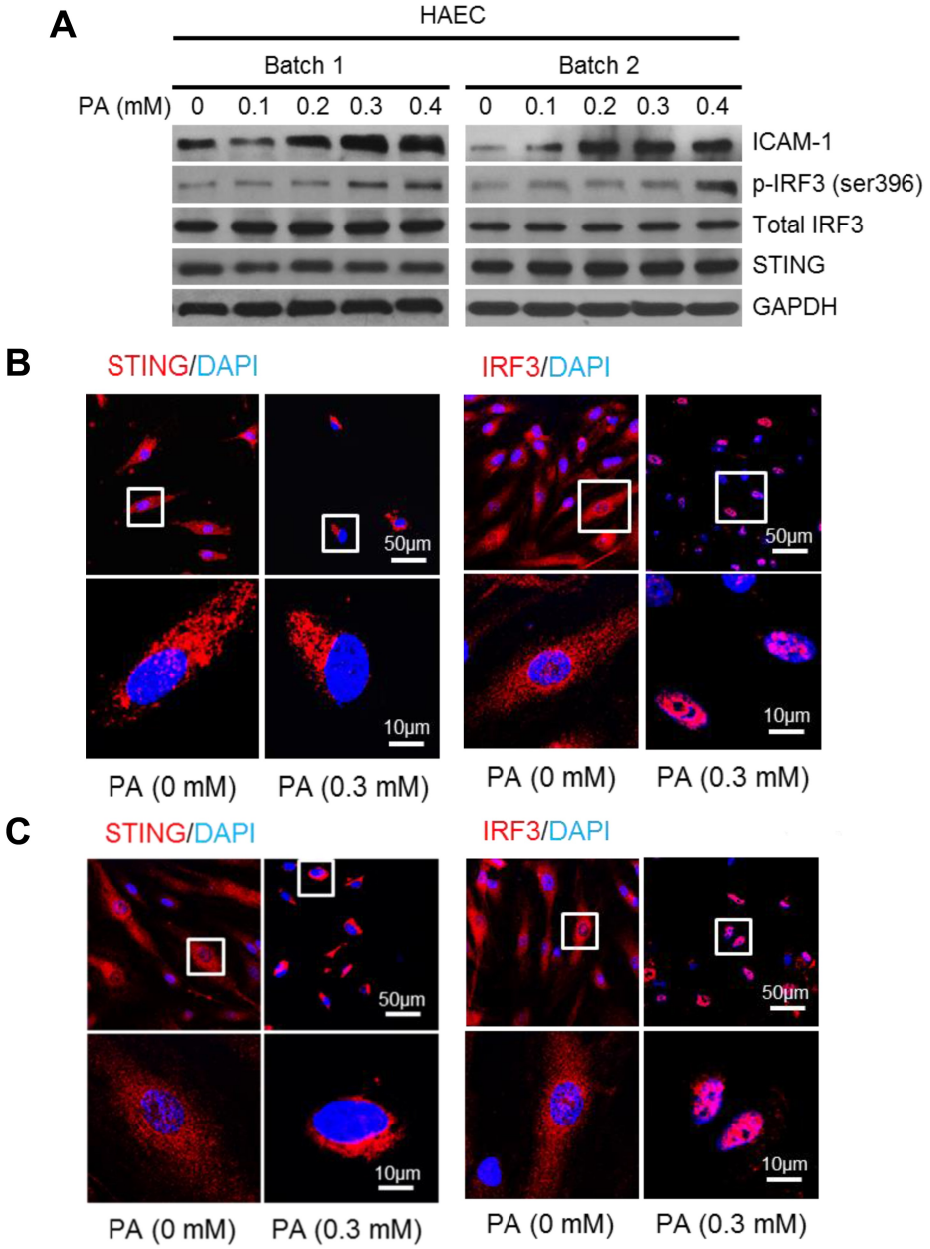
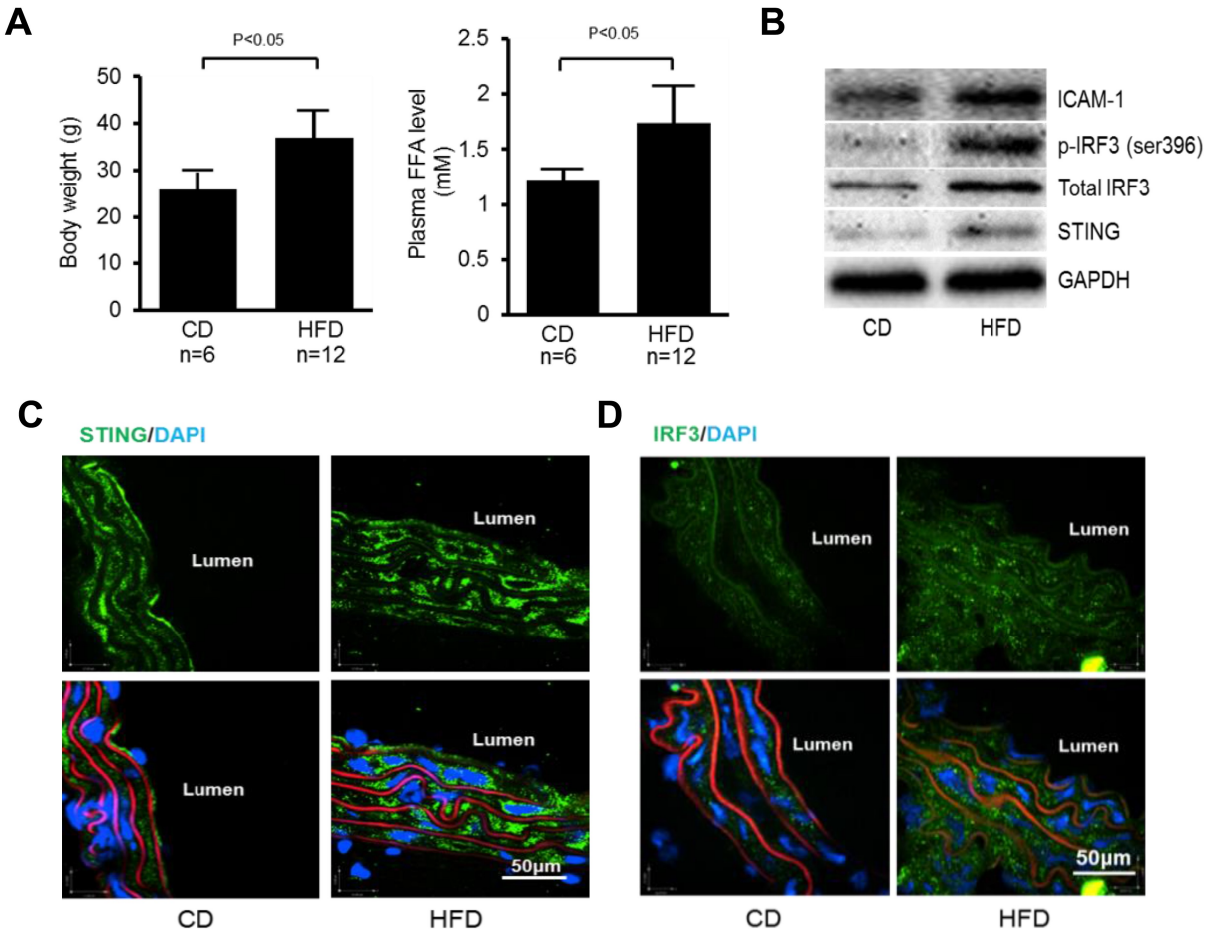

A
HAEC
Batch 1
Batch 2
PA (mM) 0 0.1 0.2 0.3 0.4 0 0.1 0.2 0.3 0.4
ICAM-1
p-IRF3 (ser396)
Total IRF3
STING
GAPDH
B
STING/DAPI
IRF3/DAPI
50μm
10μm
PA (0 mM)
PA (0.3 mM)
C
STING/DAPI
IRF3/DAPI
50μm
10μm
PA (0 mM)
PA (0.3 mM)

Figure S II


A
P<0.05
Body weight (g)
50
40
30
20
10
0
CD
n=6
HFD
n=12
P<0.05
Plasma FFA level (mM)
2.5
2
1.5
1
0.5
0
CD
n=6
HFD
n=12
B
ICAM-1
p-IRF3 (ser396)
Total IRF3
STING
GAPDH
CD
HFD
C
STING/DAPI
Lumen
Lumen
Lumen
Lumen
50μm
CD
HFD
D
IRF3/DAPI
Lumen
Lumen
Lumen
Lumen
50μm
CD
HFD